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(54) Title: CARBOHYDRATE FORMULATION (PREBIOTIC ADJUVANT) FOR ENHANCEMENT OF IMMUNE RESPONSE

(57) Abstract: A prebiotic for enhancement of an immune response, a nutritional composition for enhancement of an immune response; use of a prebiotic in the manufacture of a medicament or nutritional composition for enhancement of an immune response; use of a prebiotic in the manufacture of a medicament or nutritional composition for the prevention or treatment of measles; a method of enhancing an immune response which comprises administering an effective amount of a prebiotic; and a method of prevention or treatment of measles which comprises administering an effective amount of a prebiotic. In preferred embodiments the prebiotic comprises a fructo oligosaccharide.

Carbohydrate Formulation (prebiotic adjuvant) for Enhancement of Immune Response

The present invention relates to a carbohydrate formulation for enhancement of an immune response, a nutritional composition for enhancement of an immune response; use of a prebiotic formulation in the manufature of a medicament or nutritional composition for enhancement of an immune response; use of a prebiotic formulation in the manufacture of a medicament or nutritional composition for the improvement of an immune response to a vaccination, in particular measles vaccination, and prevention and supportive treatment of diseases *and infections e.g. bacterial,viral and parasitic*; a method of enhancing an immune response which comprises administering an effective amount of a prebiotic mixture; and a method of prevention or supportive treatment of diseases which comprises administering an effective amount of a prebiotic formulation.

Within the context of this specification the word "comprises" is taken to mean "includes, among other things". It is not intended to be construed as "consists of only".

It is well known that prebiotics comprise carbohydrates and more specifically, oligosaccharides. Furthermore it is known that they have widely been used as functional food ingredients. They resist hydrolysis by enzymes of the human digestive tract, can reach the colon undegraded and provide a carbohydrate substance particulary suited to the growth of bifidobacteria. Oligosachharides may be produced from glucose, galactose, xylose, maltose, sucrose, lactose, starch, xylan, hemicellulose, inulin, or a mixture thereof. Purified commercially available products such as fructooligosaccharides contain greater than about 95% solids in the form of oligosaccharides.

Measles is a major public health problem, infecting approximately 70 million children annually, and it is estimated that 2 million die each year from the disease itself or its complications. In addition to fever and rash, the consequences of measles include acute diarrhea or dysentery, pneumonia, encephalitis, and blindness due to acute vitamin A deficiency. Thus in developing countries, case fatality rates may reach 10-20% (Semba R.D. Clin. Infect. Dis. 1994; 19:489-499).

Measles prevention is possible by maintaining a high level of immunization through vaccination with attenuated live vaccine.

Measles vaccine is usually given at 15 months but may be given earlier (at 6-9 months of age) in areas where disease is frequently occurring and poses a threat to health and life of children. However, the response to measles vaccination at less than 12 months of age is suboptimal because infants may transplacentally acquire maternal antibodies that disappear at a variable rate. Because the seroconversion rate following immunization is not 100% and there may be some waning of immunity with time, a second immunization against measles is usually indicated.

An elevated response to early measles vaccination may therefore offer substantial and longer lasting protection until a second vaccine is administered.

The present invention addresses the problems set out above.

Remarkably, it has now been found that children fed a diet comprising a prebiotic formulation have a significantly enhanced immune response after vaccination than children fed a control diet without this prebiotic formulation.

Consequently, in a first aspect the present invention provides a composition comprising at least one prebiotic for enhancement of an immune response.

In a second aspect the invention provides use of a prebiotic in the manufature of a medicament or nutritional composition for enhancement of an immune response.

In a third aspect the invention provides use of a prebiotic or composition in the manufature of a medicament or nutritional composition for the prevention or supportive treatment of measles.

In a forth aspect the invention provides a method of enhancing an immune response which comprises administering an effective amount of a prebiotic or composition comprising at least one prebiotic.

In a fifth aspect the invention provides a method of prevention or supportive treatment of diseases such as measles which comprises administering an effective amount of a prebiotic or composition comprising at least one prebiotic.

Preferably, an embodiment of the composition is a nutritional composition which comprises at least one prebiotic.

Preferably, an embodiment of the prebiotic comprises an oligosachharide produced from glucose, galactose, xylose, maltose, sucrose, lactose, starch, xylan, hemicellulose, inulin, or a mixture thereof. More preferably the oligosaccharide comprises fructooligosaccharide. Most preferably the prebiotic comprises a mixture of fructooligosaccharide and inulin. Preferably this mixture comprises PREBIO1® or a mixture of commercially available RAFTILOSE® and RAFTILINE®.

Preferably, an embodiment of the prebiotic comprises about 50% to about 90% fructooligosaccharide. More preferably it comprises about 60% to about 80% fructooligosaccharide. Most preferably it compirses about 70% fructooligosaccharide.

Preferably, an embodiment of the prebiotic comprises about 10% to about 50% inulin. More preferably it comprises about 20% to about 40% inulin. Most preferably it compirses about 30% inulin.

Preferably, an embodiment of the composition comprises a probiotic in addition to the prebiotic. Preferably the probiotic is selected from the group which consists of Bifidobacterium bifidum and streptococcus thermophilus. Preferably the Bifidobacterium bifidum is Bifidobacterium lactis.

An advantage of the present invention is that it provides an elevated immune response after vaccination and therefore offers substantial protection until a second follow-up vaccine can be administered.

Another advantage of the present invention is that it provides an elevated response to early measles vaccination may therefore offer substantial protection against measles until a second measles vaccine is administered.

Yet another advantage of the present invention is that it may be employed to enhance an immune response, eg protection against measles, by simple consumption of food before, during, and after the vaccination period. It will be apprecieated that intravenous or subcutaneous administration of a drug requires expertise, and compared to oral administration it is not as safe, convenient or acceptable to the patient. In the light of these concerns, the invention provides the clear advantage of a nutritional and/or therapeutic product which may be administered orally.

Additional features and advantages of the present invention are described in, and will be apparent from, the description of the presently preferred embodiments which are set out below.

In an embodiment, a nutritional composition comprises a milk based cereal together with a prebiotic formulation. Preferably the milk based cereal is an infant cereal which acts as a carrier for the prebiotic formulation.

The prebiotic comprises a mixture of fructooligosaccharides and inulin in the amounts by weight of 70% fructooligosaccharides and 30% inulin.

An embodiment of the composition may comprise a source of protein and/or a source of carbohydrate and/or a source of fat.

Dietary protein is preferred as a source of protein. The dietary protein may be any suitable dietary protein; for example animal protein (such as milk protein, meat protein or egg protein); vegetable protein (such as soy protein, wheat protein, rice protein, and pea protein); a mixture of free amino acids; or a combination thereof. Milk proteins such as casein, whey proteins or soy protein or a mixture thereof are particularly preferred.

An embodiment of the composition may comprise a fat source, the fat source preferably provides about 5% to about 55% of the energy of the composition; for

example about 20% to about 50% of the energy. Lipid making up the fat source may be any suitable fat or fat mixture. For example soy oil, palm oil, coconut oil, safflower oil, sunflower oil, corn oil, canola oil, lecithins or animal fat such as milk fat may be added if desired.

An embodiment of the composition may comprise a source of carbohydrate. It preferably provides about 40% to about 80% of the energy of the composition. Any suitable carbohydrate may be used, for example sucrose, lactose, glucose, fructose, corn syrup solids, maltodextrin, or a mixture thereof.

An embodiment of the composition may comprise dietary fibre if desired. Preferably, it comprises up to about 5% of the weight of the nutritional composition. The dietary fibre may be from any suitable origin, including for example soy, pea, oat, pectin, guar gum, gum arabic, fructooligosaccharide or a mixture thereof.

Suitable vitamins and minerals may be included in the nutritional composition in an amount to meet the appropriate guidelines.

One or more food grade emulsifiers may be included in the nutritional composition if desired; for example diacetyl tartaric acid esters of mono- and di-glycerides, lecithin and mono- or di-glycerides or a mixture thereof. Similarly suitable salts and/or stabilisers may be included.

The nutritional composition for enhancing an immune response eg following measles vaccination is preferably enterally administrable; for example in the form of a powder, tablet, capsule, a liquid concentrate, solid product or a ready-to-drink beverage. If it is desired to produce a powdered nutritional formula, the homogenized mixture is transferred to a suitable drying apparatus such as a spray drier or freeze drier and converted to powder.

Alternatively, a usual food product may be enriched with the an embodiment of composition. For example, a fermented milk, a yogurt, a fresh cheese, a renneted milk, a confectionery bar, breakfast cereal flakes or bars, a drink, milk powder, soy-based product, non-milk fermented product or a nutritional supplement for

clinical nutrition. Then, the amount of the composition added is preferably at least about 0.01% by weight.

An embodiment of the composition may be included in article of confectionery, for example a sweet or sweetened beverage.

The following examples are given by way of illustration only and in no way should be construed as limiting the subject matter of the present application. Percentages and parts are by weight unless otherwise indicated.

Example 1: Nutritional Composition.

A composition was made by blending a cereal product with 4% prebiotic (70% fructooligosaccharide, 30% inulin). Its composition is indicated below:

	%
Cereal product	96%
Prebiotic	4%

Infants received 1-2 servings of this composition or cereal without the prebiotic (per serving 25g cereal and 70ml of water) per day throughout a 10 week study period. The amount of cereal consumed per day was recorded. No restrictions were made for intake of milk, solids or family food.

Remarkably, if a nutritional composition according to the invention was consumed it was found that the concentation of IgG antibody 10 weeks after a measles vaccination was significantly higher compared to consumption of a similar nutritional composition without the prebiotics. Remarkably the concentration of IgG antibodies in the blood has been found to be significantly increased when the composition comprising prebiotics was consumed. Suprisingly the level of IgG was at least 50% higher.

A double-blind randomized controlled study was conducted to examine the effects on the immune response after measles vaccination of an infant cereal with milk (Nestle) supplemented with a "prebiotic" mixture of fructo-oligosaccharides and inulin (PREBIO1®).

Eight months-old infants with mixed feeding (breast-, formula, and solids) were randomly assigned to two groups. Both groups received the cereals during a period of 10 weeks, and one group was supplemented with the PREBIOl® mixture of fructooligosaccharides and inulin (Ig per 25g cereal). Four weeks after introduction of the cereals, all infants were vaccinated with live attenuated measles vaccine (Biofarma, Indonesia). Blood was collected for IgG measles antibody measurement (Elisa; PanBio, Australia) immediately before and 6 weeks after vaccination. Growth, general health status and mild reactions after vaccination (e.g. fever, runny nose) were recorded.

Out of 50 infants enrolled, 24 infants having their diets supplemented with a composition according to the invention (S) and 25 controls not having their diets supplemented with a composition according to the invention (C) completed the study. Post-vaccination IgG antibody levels were significantly higher ($p<0.05$) in group S.

IgG antibodies increased 6.6 and 4.2 fold in groups S and C respectively ($p<0.03$). The post-vaccination IgG positivity rates were 96% (S) and 88% (C). Mild reactions were significantly more often observed in group S ($p<0.0$ 1). No differences in growth and overall health status were observed.

It was concluded that regular consumption of infant cerelas with the prebiotic composition according to the invention improved immune response eg after measles vaccination.

Example 2: Food supplement

A food supplement was prepared by mixing or blending fructooligosaccharide with inulin in the proportions by weight of about 70% fructooligosaccharide to about 30% inulin. The resulting prebiotic mixture may be added or blended with any suitable carrier, for example a fermented milk, a yogurt, a fresh cheese, a renneted milk, a confectionery bar, breakfast cereal flakes or bars, a drink, milk powder, soy-based product, non-milk fermented product or a nutritional supplement for clinical nutrition.

It should be understood that various changes and modifications to the presently preferred embodiments described herein will be apparent to those skilled in the art. Such changes and modifications can be made without departing from the spirit and scope of the present invention and without diminishing its attendant advantages. It is therefore intended that such changes and modifications be covered by the appended claims.

Claims

1. A composition for use in the treatment and/or prevention of measles, characterized in that it contains at least one prebiotic.

2. The composition according to claim 1, wherein the prebiotic comprises an oligosaccharide produced from glucose, xylose, maltose, sucrose, lactose, starch, xylan, hemicellulose, inulin or a mixture thereof.

3. The composition according to claim 1 or 2, wherein the prebiotic comprises fructooligosaccharides, inulin or a mixture thereof.

4. The composition according to any of the preceding claims, wherein the prebiotic is contained therein in an amount of from about 20 to 80 % by weight.

5. The composition according to any of the preceding claims, comprising about 60 to 80 % by weight fructooligosaccharides and about 20 to 40 % by weight inulin.

6. The composition according to any of the preceding claims comprising a probiotic.

7. The composition according to claim 6, wherein the probiotic is selected from the genus Lactobacillus, Bifidobacterium and/or Streptococcus.

8. The composition according to claim 7, wherein the probiotic is selected from Bifidobacterium bifidum and/or Streptococcus thermophilus.

9. The composition according to any of the claims, which is a food composition selected from milk, yogurt, curd, cheese, fermented milks, milk based fermented products, ice-

cream, fermented cereal based products, milk based powders, infant formulae, pet food or a pharmaceutical composition selected from tablets, liquid suspensions, dried oral supplement, wet oral supplement, dry tube feeding or wet tube-feeding.

10. Use of a composition according to any of the preceding claims for preparing a carrier for treating or preventing measles.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 01/01627

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/715

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	FIRMANSYAH ET AL: "Improved humoral response to measles vaccine in infants receiving infant cereal with fructooligosaccharides" JOURNAL OF PEDIATRIC GASTROENTEROLOGY AND NUTRITION, vol. 31, no. suppl2, - 7 August 2000 (2000-08-07) page s134 XP001000242 abstract	1,2
A	WO 87 02679 A (UNIV AUSTRALIAN) 7 May 1987 (1987-05-07) the whole document	1-10
A	EP 0 692 252 A (RAFFINERIE TIRLEMONTOISE SA) 17 January 1996 (1996-01-17) the whole document	1-10

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
10 July 2001	25/07/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Engl, B

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INTERNATIONAL SEARCH REPORT

In. ...tional Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZIEMER C J ET AL: "AN OVERVIEW OF PROBIOTICS, PREBIOTICS AND SYNBIOTICS IN THE FUNCTIONAL FOOD CONCEPT: PERSPECTIVES AND FUTURE STRATEGIES" INTERNATIONAL DAIRY JOURNAL,ELSEVIER APPLIED SCIENCE, BARKING,,GB, vol. 8, May 1998 (1998-05), pages 473-479, XP000972201 ISSN: 0958-6946 the whole document ---	1-10
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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